1/2/41

January 2, 1941

Dr. Warren Weaver
The Rockefeller Foundation
49 West 49th Street
New York, New York

Dear Warren:

I am writing now to ask your advice about our research program in immunology.

First let me say that the work in organic chemistry and structural chemistry is going along well. Zechmeister, with two post-doctorate assistants and four graduate students, is making headway on several problems. He has fitted into our department satisfactorily, and there has been no trouble at all of the sort that sometimes accompanies the appointment of a European professor. Carl Niemann is working as hard as ever and as effectively. The molecular structure program is booming along. The crystal structure of alanine, now completed, has turned out to be especially interesting. In this crystal for the first time the positions of hydrogen atoms were verified by the x-ray data. We are now beginning to get good x-ray photographs of large polypeptides, with fifty or one hundred amino acid residues per molecule, and I think that we shall succeed in making complete structure determinations of these. At present we are studying polyglycines, and Dr. Edsall, who is here this year as Guggenheim Fellow, is now preparing some polypeptides containing both glycine and alanine. I am confident that this method of attack, using synthetic polypeptides of known amino acid composition, which might be called artificial proteins, will lead ultimately to the solution of the protein problem.

For about six months Dr. Dan Campbell, Rockefeller Fellow, and Dr. David Pressman have been working with me on experimental problems in

immunology. This work has moved along slowly, because of its nature, but I am pleased with the results obtained. It was fortunate for us that Dr. Campbell was here. Dr. Pressman, an organic chemist trained here, was supported in part from the Rockefeller Foundation and in part from the Noyes Fund of the Institute. All together we have used about $2000 of this year's Rockefeller budget for the work in immunology. The researches completed or under way are the following.

1. Serological reactions with simple substances containing two or more haptenic groups. We have shown, verifying the work of Landsteiner and van der Scheer, that simple substances containing two or more haptenic groups will give the precipitin reaction with homologous antiserum and produce shock in sensitized guinea pigs. Hooker and Boyd had said that they could not obtain these reactions. We have made quantitative studies of the precipitin reaction, determining the amount of antibody nitrogen precipitated by the micro-Kjeldahl method. So far we have positive results for six substances involving arsanilic acid residues attached to resorcinol or phloroglucinol. These results are described in a paper which Campbell, Pressman, and I are publishing in the Proceedings of the National Academy of Science. The production of a precipitate by simple substances containing two or more groups is, I think, a very strong argument for the framework theory of antibody-antigen precipitates.

2. The manufacture of antibodies in vitro from normal serum proteins. In my paper I suggested that it should be possible to manufacture antibodies in vitro by denaturing serum proteins and slowly renaturing them in the presence of antigen. Experiments along these lines have been carried out, using three different fractions of beef globulin and the dye methyl blue as antigen. It was found that after denaturation of the protein with alkali

or by heat slow renaturation in the presence of the dye produced a protein solution which combined with the dye much more strongly than protein solutions obtained in other ways, such as by rapid renaturation in presence of dye or by various treatments in absence of dye. Similar experiments were started here with bacteria as antigen, and Dr. Campbell plans to continue them in Chicago. Further work is needed to test the specificity of the artificially produced antigens, but the results obtained already are positive.

3. A quantitative study of sera homologous to antigens containing two different haptenic groups. Landsteiner reported experiments suggesting that no antibody molecules of type A'-B' were present in the serum produced by antigens containing two different groups A and B. This result is incompatible with my theory or any theory involving bivalent or multivalent antibody molecules. With the cooperation of Dr. Landsteiner, we have been carrying out quantitative studies of his sera, and have obtained results which support the conclusion opposite to that reached by him.

4. The theory that agglutination is the result of the clasping together of cells by bivalent antibody molecules requires that inhibition of agglutination occur on treatment of cells by a sufficiently strong agglutinin solution. Hooker and Boyd have reported failure to produce agglutination in erythrocytes and argue accordingly against the bivalent antibody theory. We are carrying out agglutination experiments using antiarsanilic acid serum as agglutinin and erythrocytes treated with diazotized arsanilic acid as the cells.

There are many other experiments which we plan to carry out. These include the quantitative study of antibody-antigen composition of precipitates, the determination of equilibrium constants and heats of reactions by physical-chemical methods, especially by the use of various haptens for the

study of soluble complexes, the separation of antibodies of different kinds by combination with charged haptens and subsequent electrophoresis, the investigation of the order of the reaction of antibody production by quantitative studies of serum titer for large numbers of rabbits immunized by different amounts of antigen, etc. We are handicapped at present by our inability to determine the amounts of antigens, especially those containing arsenic, in small precipitates with sufficient accuracy. This problem can be solved easily by the use of radioactive arsenic (the isotope As^{74} made from germanium by deuteron bombardment, with sixteen-day half life), and Edwin McMillan has arranged for this to be made for us in Berkeley when we are in a position to use it effectively.

I feel enthusiastic about the program of research in immunology, and I would like to carry it on vigorously for some time in the future. In addition to the experiments mentioned above, we have many others under consideration, some of which have been suggested by the immunologists who have written to me since the appearance of my paper. I have, in fact, been carrying on a rather lively correspondence with immunologists of the country, especially with Dr. Boyd in Boston, who is strongly opposed to my theory in so far as it involves bivalent antibodies. I find that immunological work is laborious, and that it moves along slowly unless a suitable number of assistants are available. I am afraid that it would not be possible to take from our present funds enough money to permit the work to be done effectively--we got along this year because of our accidental good fortune in having Dan Campbell here.

The program which I have in mind would involve the expenditure of about $20,000 per year, with the following budget:

Research Fellow, Immunology	$3000.00
Research Fellow, Organic Chemistry	2400.00
Research Fellow, Physical Chemistry	2000.00
Assistant, Immunology	1500.00
Assistant, Organic Chemistry	1200.00
Assistants, Microanalysis and Radioactivity	1400.00
Apparatus, supplies, animals	7500.00
Expenses of visiting professor	1000.00
Total	$20,000.00

As Research Fellow in Immunology I would like to have Dr. Dan Campbell, at present Assistant Professor of Bacteriology and Immunology at the University of Chicago. He is a good technician in the field, an industrious man who works steadily day after day, and a man with interest in chemistry and with an open mind regarding science. I doubt that a better man could be found for this appointment. As Research Fellow in Organic Chemistry I would suggest Dr. David Pressman, who has been carrying on this work this year, and has become deeply interested in immunology. He has been helped in the preparation of organic substances this year by two seniors in chemistry, the two best men in our senior class. Both are American-born Japanese. I think that one might be kept on next year full time as assistant in organic chemistry. The item of $7500.00 for apparatus, supplies, animals would permit us to use the large number of animals required for some of our projected researches, and should permit also the construction of a Tiselius apparatus for the

electrophoretic separation of antibody fractions by the suggested method of combination with charged haptens, and for other investigations. I have included the item $1000.00 for expenses of a visiting professor. It seems to me that one of the best ways of assuring that we were making an effective attack on immunological problems would be to bring here each year some authority in the field, for discussion and collaboration. This would be of value also to the people here in biology.

I would like to be able to plan to carry on this program over a five-year period.

Would you please tell me whether you think that it would be worth while for an application along these lines to be submitted to the Rockefeller Foundation and if so in what respects the application should deviate from the outline given in this letter. I would like our attack on the problem to be intensive enough to be effective, and the program which I have described above is, I think, just about right.

With best regards, I am

Sincerely yours,

Linus Pauling

LP:jr